A STUDY OF THE HISTOLOGIC CHANGES INDUCED IN LEAVES BY CERTAIN LEAF-SPOTTING FUNGI

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H. S. CUNNINGHAM

INTRODUCTION

Just why many necrogenic fungi parasitic upon the leaves of plants make only a limited growth resulting in definite lesions known as "spots" has not been satisfactorily explained. So far as the writer is aware there is recorded in literature no comprehensive investigation on the subject. In the many papers dealing with leaf-spotting fungi certain suscept reactions which may have some limiting influence upon the growth of the pathogene have occasionally been recorded. No doubt in many cases the reasons for such limitation of growth are purely physiological in character and have to do with the biochemical relation of pathogene and suscept. In a few cases there have been reported certain histologic changes in the suscept brought about as a result of the invasion of a necrogenic pathogene. Such histologic changes involve the revival of a meristematic condition in certain cells of the mesophyll resulting in the formation of new cells and an enlargement of these cells as well as of cells already in existence, with the ultimate production of a definite cicatrice separating the diseased portion from the healthy. Such a condition is reported as being more or less common in the leaves of higher plants when subjected to mechanical injury (Wylie, 21).

As a result of recently published work on the response of leaves to wounding and at the suggestion of Professor H. H. Whetzel, the present investigation was undertaken. The purpose of these studies has been to determine to what extent similar histologic changes are induced by leaf spotting fungi and whether such suscept responses play a major rôle in the restriction of their necrogenic activities.

[1] This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames, under whose direction the work has been done.

It was deemed desirable to study as many different leaf-spot diseases as possible in order to arrive at any conclusions of general application. It was also necessary in each case to study a large number of sections to be sure of the true nature of the changes which take place. These changes were found to vary greatly with the proximity of the spot to veins and islet borders. In all probability the age of the leaf at the time of infection and also environmental factors play an important part in the type of suscept reaction. In the case of wounding, the evidence of other workers goes to show that the moisture content of the atmosphere has a marked influence upon the type of healing tissue formed (Blackman and Matthaei, 1; Samuel, 17). It was also thought advisable to study, in a few cases at least, the response of the suscept to artificial wounding.

All of the material used in these investigations was collected in the vicinity of Ithaca, New York. Specimens of the diseased material, together with representative slides, have been deposited in the herbarium of the Department of Plant Pathology, Cornell University.

HISTORICAL REVIEW

While there are numerous references in literature to the healing of wounds in woody stems and the histologic changes resulting in callus formation, there has been published comparatively little regarding the reaction of leaves to mechanical injury and still less with respect to their reaction to invasion by necrogenic pathogenes.

Such references are scattered through various journals and in many cases are merely short paragraphs in a publication dealing primarily with the life history of the pathogene involved. This being the case, the author makes no claim to having exhaustively searched such literature but feels that the more important papers dealing with the subject have been consulted.

Bretfeld (2) states that in *Camellia japonica* wounds are healed simply by drying of the wound surface but that in *Bryophyllum sp.*, for example, there is often formed a large and peculiar periderm and that in general the arrangement of this periderm is parallel to the edge of the wound.

According to Pierce (14) the shot-hole lesion in leaves of almond caused by *Cercospora circumcissa* is bounded by partially dead and thickened tissues.

Frank (5, p. 63–67) states that wounds made with a scalpel in the leaves of *Leucojum vernum* are healed by callus formation, while insect wounds in the leaves of *Cornus sanguinea* are healed by the production of wound cork. In the former case the healing tissue consists of a mass of enlarged cells with suberized walls while in the latter case a definite periderm is formed.

Massart (11) describes and figures a cicatrice in a wounded leaf of *Nuphar sp.* formed about the lacunae. He says that the age of the tissue is important in determining the extent of cicatrice formation.

Miehe (13), studying wounded leaves of *Tradescantia sp.*, reports that the bared epidermal cells grow out and form a callus which at completion forms a cork layer.

Blackman and Matthaei (1) removed leaves of *Prunus laurocerasus* var. *rotundifolia* and placed the petioles in a beaker of water. When these leaves were wounded no healing tissue was formed. If, however, a sufficient number of cells were killed about the edge of the wound, an abscission layer was formed. The wounded portion eventually dropped out and the walls of the callus cells became cuticularized. Leaves wounded on the plants by cutting formed an abscission layer very quickly. The wounded portion did not fall out but division of the newly formed cells continued until a periderm of several layers of cork resulted.

Van Beusekom (18) noted that insect wounds on the tips of leaves of *Gnetum gnemon* brought about the formation of adventitious buds, and when the swollen tips were sectioned they showed multiplication and enlargement of the cells of the spongy parenchyma.

Wyneken (24) studied wound reactions in leaves in a large number of species of the higher plants and found that in some species a definite wound cork was formed. In such cases the outside layer of cells always had suberized walls, while in some cases lignification appeared in the walls of those cells lying next the suberized layer. In other species a callus composed of irregular, thick-walled cells was formed about the wound.

Higgins (7), working with *Coccomyces sp.* on species of *Prunus,* found that there was considerable variation in the deciduous character of the lesions in different species and even in the same species. He states that the separation is due to the abrupt enlargement of a layer of cells at some distance from the ends of the mycelium. The enlarged cells lose their chloroplasts and nuclei, and only a thin layer of protoplasm lines the walls.

A definite case of a barrier being laid down is given by Hesler (6), who worked with *Sphaeropsis malorum* on leaves of *Pyrus malus.* Where the lesion is not bounded by veins it is surrounded by a plate of cells which limits, for a time at least, the advance of the pathogene. The apparent stimulation results eventually in hyperplasia of the spongy tissue. The diseased cells gave a positive test for suberin.

Wylie (21), who severely wounded leaves of several species of higher plants, found that healing takes place quickly and the leaf continues to function. In a later paper (22), he discusses the structure of the healing tissues in species of widely different habitats. He found that this tissue is largely

formed by the development of new cells resulting from mitoses which establish walls parallel to the wounded edge of the leaf. All cell-layers of the blade share in this work, including the epidermis in case its cells are large. In a still later paper (23) he describes the healing process as embracing first the formation of a pseudo-cicatrice as a result of the death and collapse of exposed cells along the wounded edge, which is followed by the formation of the cicatrice proper.

Woit (20) studied wounded leaves in a number of species belonging to different families of the higher plants and found that many cells in full grown leaves are stimulated to division and new growth. Cells of the collenchyma, conductive tissue, and hairs are often active in the wound reaction. In a moist atmosphere callus is formed, while a freely exposed wound usually develops wound cork only.

In a study of *Prunus amygdalus,* attacked by *Clasterosporium carpophilum, Samuel* (17) found that the infected tissues in young leaves are invariably abscissed. In the older leaves an abscission layer is formed but the infected tissues do not always fall out. The abscission layer begins with the swelling of cells in a narrow zone at some distance from the margin of the invaded area. Both palisade and spongy parenchyma cells are involved and complete occlusion of the intercellular spaces results. Where the invaded portion is abscissed, suberization of the walls of the cells along the absciss line occurs soon after the cuticle ruptures. Subsequent divisions of the meristematic layer result in the formation of a few layers of brick-shaped cells which become suberized and slightly lignified. Where abscission does not occur the initial changes are similar, but in the later stages there is lignification of the walls of the cells on the inner side of the occluded zone and a suberization of the walls of the meristematically formed cells.

McWhorter (12, p. 19–21) describes the formation of a meristem in the leaves of *Carica papaya* infected with *Cladosporium papayae.* This meristem acts like a cork cambium, cutting off cork cells. The fungus is able to penetrate these cork cells to a limited extent.

It is evident from these records that mechanically wounded leaves of many plants respond by histologic modification of adjacent tissues. They also indicate that, at least in certain fungous diseases, similar healing-out modifications take place in the tissues about the lesion. However, one would be led to conclude from the very limited number of such cases recorded that such a response to the activity of pathogenes is relatively rare.

METHODS

As the material was collected small segments were removed from the diseased leaves by means of a razor blade. These segments included both

diseased and healthy tissue. When the lesions were small the portion removed from the leaf included the whole of the necrotic area. In the case of the larger lesions this procedure was not possible, as the size of the segment was necessarily limited by the difficulties of fixation. As far as possible only mature leaves with well developed lesions were used in these studies, several segments being taken from each of a number of infected leaves.

Immediately the segments were removed from the leaf they were placed in shell vials containing medium chromo-acetic fixing solution (3). In the case of some material, a vacuum-pump was used to insure rapid removal of air from the tissues. Other material was merely forced under the liquid by means of a wad of cheesecloth placed on top of the segments in the vial. The killing was perfectly satisfactory by both methods. The material remained in the fixing solution for from 24 to 36 hours and on removal was washed in running water for an equal length of time, after which it was imbedded in paraffin (53°–55° F.) in the usual manner.

Whenever time would permit, freehand sections were also made of the fresh material. At this time studies were made on the extent of the mycelial development, and microchemical tests were applied to determine the nature of the changes in the walls and contents of those cells lying at or near the edge of the lesion. In the study of freehand sections of freshly collected material cotton blue was used as a mycelial stain. This proved satisfactory in some leaves but was not so effective with others owing to the heavy staining of the chloroplasts. Tests for lignified tissue were made with phloroglucin and hydrochloric acid. The presence of cutin and suberin was determined by staining with Sudan III. This was done by boiling the sections in a drop of stain on the slide, washing in water, and mounting in glycerin for examination. The ferric-chloride test was used to determine the presence of tannins.

These tests were repeated with sections made from material imbedded in paraffin. In testing for suberin it was not possible to boil the stain on the slide after the sections had been attached with albumen fixative. Good results were obtained by placing the slides in a Coplin jar containing the stain and keeping this at a temperature of 40° C. for 36 hours. The slides were then thoroughly rinsed in water and mounted in glycerin for examination.

In addition to cotton blue as a mycelial stain, both Durand's (4) and Vaughan's (19) staining methods were used. None of the methods mentioned proved entirely satisfactory in all cases. For the purpose of general histologic studies, sections were stained on the slide in Delafield's haemotoxylin; safranin; Delafield's haemotoxylin and safranin combined; and in

crystal violet combined with erythrosin (8). The latter combination gave a beautiful contrast and was particularly valuable in detecting lignified tissue. In many cases it also proved valuable in staining the mycelium.

Some of the plates were prepared with the aid of a camera lucida, this being used merely to get the proportions and the bare outline of cells. The remainder of the plates were prepared by inking in the cell walls on an enlargement made from a photomicrograph. The enlargement was then treated with a solution of potassium cyanide, followed by a solution of iodine-potassium iodide, after which it was thoroughly washed and dried. This treatment removed the image and resulted in a plain black and white drawing. With both methods the details were filled in freehand. In all cases the details shown in the plates represent the author's interpretation of the condition existing in the cells as gathered from the study of a large number of sections stained in various ways.

Where artificial wounding was practiced the wounds were made on mature leaves by means of a common leather punch making a circular hole 2 mm. in diameter. In this way a clean-cut wound was obtained. As far as possible, care was taken to avoid cutting into or near the larger veins. Several wounds were made in a single leaf, the number depending somewhat upon the size of the leaf. The wounded leaves were allowed to remain on the plant for a period of from 18 to 21 days. At the end of that period they were collected and segments removed, treated, and studied in the same manner as those from diseased leaves.

PRESENTATION OF DATA

The results of the present investigation are presented under two headings based on the type of histologic changes which result from infection by the various pathogenes on the suscepts dealt with in this paper.

In practically every case the leaf-spot lesion consists of a clearly defined central dead area surrounded by a zone of varying width and coloration in which the cells are still alive but diseased. Following the terminology used by Whetzel in his mimeographed text on symptomatology, this central region will be designated the "holonecrotic" region or area, while the marginal zone will be referred to as the "plesionecrotic" zone or region.

Unless otherwise stated, the mesophyll cells are of the usual thin-walled type containing nuclei and chloroplasts in a peripheral layer of cytoplasm. The epidermal cells also have a thin layer of cytoplasm about the walls, and nuclei are present.

Diseases in which a Definite Cicatrice is formed about the Margin of the Lesion

Three species of *Coccomyces* and one species of *Mycosphaerella,* all of which are parasitic on members of the family Rosaceae, together with one species of *Cercospora* parasitic on *Beta vulgaris,* were found to cause histologic changes of the type under discussion.

The term "cicatrice" as used in this paper is applied to a band of healing tissue about the lesion or wound, the cells of which have been derived by division and enlargement of the mesophyll cells. The epidermal cells are also frequently involved. This cicatrice ordinarily consists of two parts, the outer part which is a typical wound periderm, and the inner part which lies between the wound periderm and the normal tissue. This latter part is composed of somewhat modified cells evidently not derived from the activities of the wound phellogen.

Lesions produced by Coccomyces prunophorae Higgins on Prunus domestica L. In the healthy portions of the leaf of *Prunus domestica* the upper epidermal cells are large and somewhat irregular in outline. Both the inner and outer walls are convex but this is more marked in the case of the inner walls. The outer walls are noticeably thickened as are also the radial ones, while the inner are comparatively thin. Except that the cells are somewhat smaller, the structure of the lower epidermis is very similar to that of the upper. The palisade parenchyma is well-defined and consists of two or even three layers of elongated narrow cells. In a cross-section of the leaf the upper layer appears unbroken by intercellular spaces. The lower layer, however, is less compact, and intercellular spaces are evident between many of the cells. The cells of the spongy parenchyma are more or less irregular in size and shape and are rather loosely arranged. In the immediate vicinity of the larger veins the palisade parenchyma is replaced by enlarged, circular to irregularly shaped, thick-walled parenchyma cells, while the region of the spongy parenchyma is occupied by the principal bundle tissues with strengthening cells below. Where smaller veins are present the palisade parenchyma is still intact, but that portion of the spongy parenchyma not occupied by the bundle is filled with somewhat small, thick-walled parenchyma cells.

The necrotic lesions caused by the fungus vary considerably in size and appearance depending upon the age of the lesion and the extent of the infection. The smaller lesions are more or less circular in outline, the dark-brown holonecrotic area being surrounded by a narrow yellowish-green plesionecrotic zone. Where the lesions are numerous they frequently coalesce to form large, irregular areas. In the older spots the diseased portion

may drop completely out, leaving a clean-cut margin. This margin may or may not be thicker than the normal leaf.

A cross-section made through one of these lesions reveals the presence of a very definite cicatrice at the margin (Fig. 1). Throughout the whole

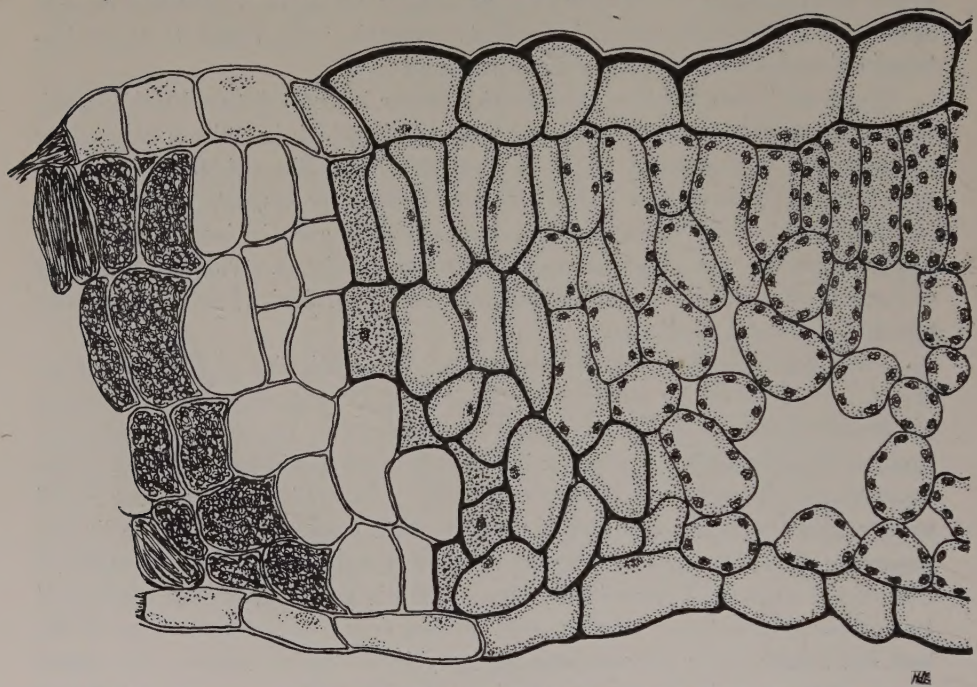

FIG. 1. Cross section through the edge of a lesion on a leaf of *Prunus domestica* caused by *Coccomyces prunophorae*. ×428.

region occupied by the cicatrice there has been division and multiplication of the cells of both the palisade and spongy parenchyma which has resulted in a band of tissue without intercellular spaces. The wound periderm is several cells in width. The phellem is made up of a mass of large cells whose thick walls are not only suberized but lignified as well. The cells lying nearest the lesion are filled with a dense granular substance resembling tannin, while the remaining cells are apparently devoid of contents. The epidermal cells of this layer also have suberized walls. Both a phellogen and phelloderm are present in the wound periderm. The cells comprising these two layers are thick-walled but this thickening is entirely cellulose in nature. Chloroplasts are absent from these cells. The phellogen consists of a single layer of cells which are filled with dense protoplasm, while the cells of the phelloderm contain only a peripheral layer. Nuclei are plainly visible in the cells of both of these layers. The inner part of the cicatrice is com-

posed of somewhat enlarged, thin-walled living cells, with only a few chloroplasts present.

All efforts failed to demonstrate the presence of the pathogene beyond the edge of the cicatrice.

The reaction to the leaf to wounding (Fig. 2) is not unlike that induced

FIG. 2. Cross section through the edge of a wound on a leaf of *Prunus domestica* showing the cicatrice formed under these conditions. ×428.

by the pathogene in question. There are, however, certain differences which seem to be characteristic. The principal difference appears in the cells of the phellem layer. In the case of wounding, the cells are somewhat smaller and the radial arrangement is more definite. The leaf in the region of the cicatrice is usually somewhat thicker than normal. The cicatrice is formed at some little distance from the edge of the wound. At the edge of the wound the cells have entirely collapsed and this condition becomes less evident as we approach the cicatrice. Many of these collapsed cells, both in the palisade and in the spongy parenchyma, are more or less filled with the tannin-like substance already referred to.

Lesions produced by Coccomyces lutescens Higgins on Prunus virginiana L. The structure of the healthy leaf of *Prunus virginiana* is very similar to that of *Prunus domestica*. The leaf of the former species, however,

is somewhat thinner, and at intervals resin cavities are formed in the upper part of the palisade parenchyma.

The lesions on this suscept are also very similar in appearance to those on *Prunus domestica* and need no further description.

When viewed in cross-section the type of cicatrice is seen to differ in some respects from that described on *Prunus domestica.* At the edge next the lesion the leaf is fully one-third thicker than normal and gradually decreases in thickness to the point where the cicatrice merges into healthy leaf tissue. Throughout the whole cicatrice there has been a multiplication and division of the cells of the spongy parenchyma and also of the lower palisade cells which has resulted in the occlusion of the intercellular spaces. The wound periderm is of considerable width. The phellem layer, which is several cells wide, is made up of much enlarged cells, more or less irregular in shape, whose walls are lignified as well as suberized. While they are not so regular in their arrangement as are the phellem cells in the wound periderm in *Prunus domestica,* they evidently have their origin in the phellogen. The epidermal cells in this layer are slightly enlarged and their walls have also become suberized. The remainder of the wound periderm is made up of thick-walled cells. The outer one or two rows lying next the phellem constitute the phellogen and are filled with protoplasm while the remaining rows contain only a peripheral layer. No chloroplasts are present in these cells. The inner layers of the cicatrice is made up of thin-walled living cells. Those nearest the wound periderm contain no chloroplasts while the number of chloroplasts is relatively few in the cells adjacent to the normal tissue. Dead cells, remnants of the diseased area, may or may not be found clinging to the outer edge of the phellem. No trace of the pathogene could be found beyond the cicatrice.

The cicatrice which is formed as a result of wounding is so similar to that formed on *Prunus domestica* under similar conditions that little further description is necessary. The chief difference lies in the fact that it is not so extensive, there being at most only two layers of cells in the phellem and these very much enlarged.

Lesions produced by Coccomyces hiemalis Higgins on Prunus cerasus L. With certain minor differences the general structure of the leaf of *Prunus cerasus,* as studied in cross section, is similar to that of the *Prunus sp.* already described. The cells of both the lower and upper epidermis are irregular in size and shape. While some of the upper epidermal cells contain the usual thin layer of protoplasm, many of them are filled with a dense substance which stains deeply with haemotoxylin. An occasional cell of the lower epidermis is also filled with this same substance.

The type of lesion found on this suscept is very similar to that described on *Prunus domestica.* A study of a cross-section made through one of these

lesions shows a cicatrice formed at the margin which is almost identical with that described on *Prunus domestica*. Here we find the same phellem layer of enlarged cells having suberized and lignified walls and apparently lacking in cell contents. Between this layer and the normal tissue there is a layer of thick-walled living cells.

Wounding of the leaf of this suscept results in the formation of a cicatrice so similar in its general characters to that formed on *Prunus domestica* under the same conditions that no detailed description need be given.

Lesions produced by Coccomyces hiemalis Higgins on Prunus avium L. The structure of the healthy leaf of this suscept is essentially the same as that of *Prunus cerasus* and the type of lesion produced by the pathogene is the same as that produced by the other species of *Coccomyces* on the suscepts studied.

As in the case of the other species of *Prunus* dealt with in this paper, there is a definite cicatrice formed at the edge of the lesion. A study of a cross-section through this formation shows that in its general structure it is similar to that already described on *Prunus domestica*.

Wounded leaves formed a cicatrice in all respects like that formed on the other species of *Prunus* when wounding was practiced.

Lesions produced by Mycosphaerella sentina (Fr.) Schr. on Pyrus communis L. In the healthy leaf of *Pyrus communis* the lower epidermis is very much like the upper in its general characters. The outer cell walls of the latter, however, are somewhat thicker than are the outer cell walls of the former. In both cases occasional cells are partly filled with a dense staining material. The palisade parenchyma consists of two layers of closely packed cells, those of the upper layer being much longer than those of the lower. The cells of the spongy parenchyma are variable as to size and shape and are loosely arranged.

The lesions on the leaf are oval to irregular in outline, are entirely holonecrotic in character, and are brown in color with a grayish-white center. The margin is somewhat darker in color than is the main body of the lesion; is slightly raised and sharply defined.

At the edge of the holonecrotic area there is a very definite cicatrice laid down. When viewed in cross-section this is seen to be similar to that described on *Prunus domestica*. The outer edge of the cicatrice ends abruptly in normal tissue, and by none of the methods used was it possible to find any evidence of the pathogene beyond this barrier.

Cross-sections of leaves artificially wounded reveal the formation of a cicatrice at some little distance from the edge of the wound. The intervening cells are dead and in some cases the epidermis has apparently folded over the edge of the wound. In all respects this cicatrice is similar to that formed at the edge of the lesion caused by the pathogene.

Lesions produced by Cercospora beticola Sacc. on Beta vulgaris L. The healthy leaf of *Beta vulgaris* as seen in cross-section is rather open in structure. The upper epidermis is composed of cells which are more or less irregular in outline. The outer and radial walls are comparatively straight but the inner walls tend to curve inward, at times even forming an obtuse angle. The outer walls are considerably thickened, the radial walls somewhat less so, while the inner walls are only slightly thickened. The cells of the lower epidermis are somewhat different from those of the upper, being slightly longer, narrower, and with the inner wall more parallel to the outer. Both the outer and radial walls are noticeably thickened. The palisade parenchyma is very open in structure. Large intercellular spaces are present, and in cross section of the leaf the cells appear in single rows, or at most in groups of two or three, each row comprising two or three elongated cells placed end to end. In the material sectioned no large veins were present, but in the presence of small veins the structure of the palisade parenchyma was unaltered. The spongy parenchyma is also very open in structure. While the cells are a bit more irregular in shape and arrangement, this tissue does not differ essentially from the palisade parenchyma.

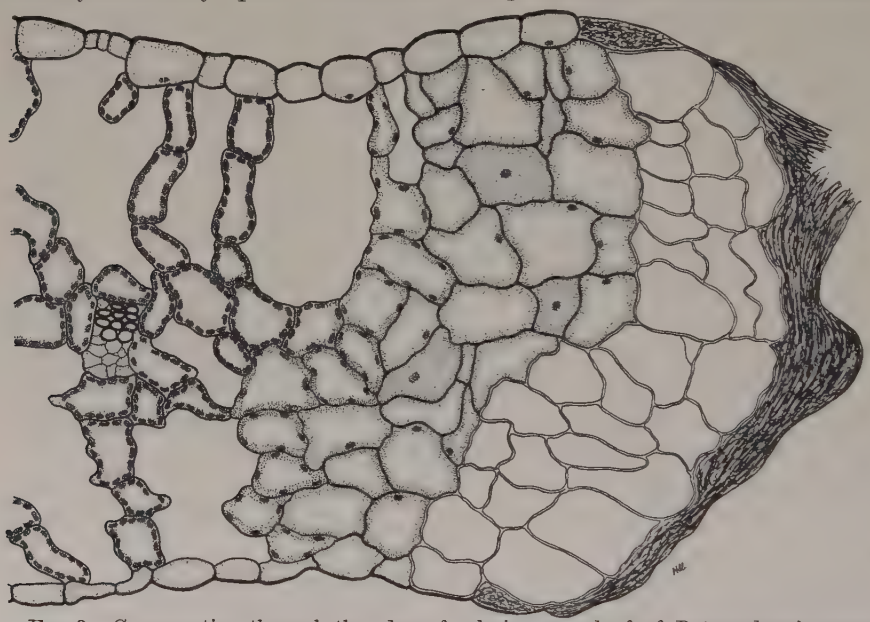

FIG. 3. Cross section through the edge of a lesion on a leaf of *Beta vulgaris* caused by *Cercospora beticola*. ×225.

The infected areas on the leaves appear as spots of varying size, more or less regular in outline, holonecrotic in character, the center of which is sunken and grayish-white in color, surrounded by a purplish-red margin.

A cross-section of the leaf made through one of these lesions (Fig. 3) shows that there has been a marked change in the character of the tissue adjacent to the sunken area. For some distance back from this region there has evidently been renewed meristematic activity in the cells of the mesophyll which has resulted in complete occlusion of the intercellular spaces and the formation of a definite cicatrice. In general the region occupied by the cicatrice is somewhat thinner than that of the healthy portion of the leaf, although there was considerable variation in this respect in the material examined. The variation may possibly have been due

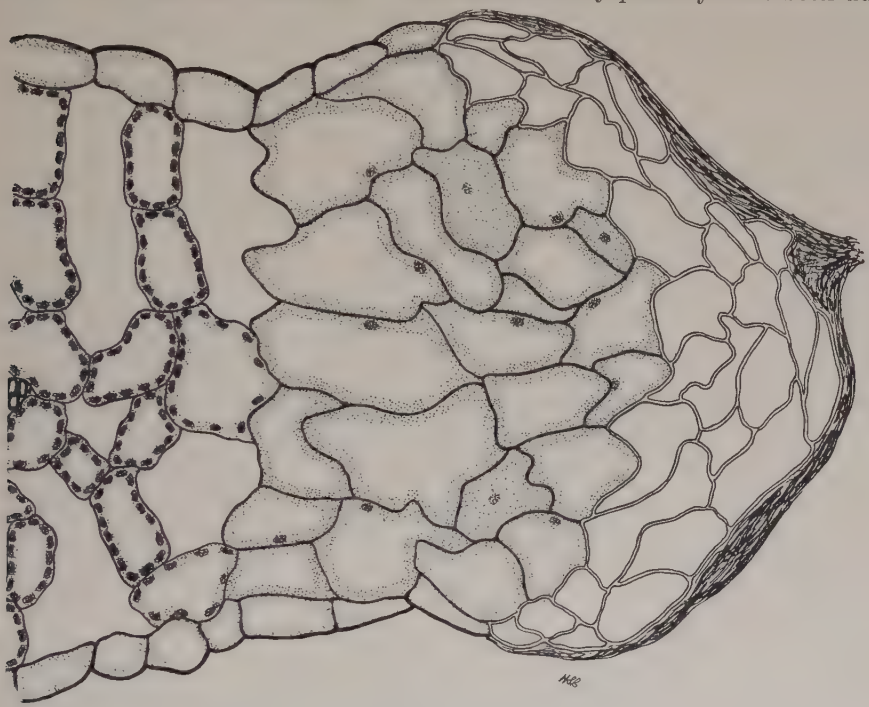

FIG. 4. Cross section through the edge of a wound on a leaf of *Beta vulgaris* showing the cicatrice formed under these conditions. ×300.

to differences in the maturity of the wounded leaves. The cicatrice does not differ essentially in character from those already described on species of *Prunus*. The cells, however, are larger and more irregular in shape, while the thickening of the walls is not so pronounced. There is a mass of dead collapsed cells attached to the outer edge of the phellem layer. All efforts failed to demonstrate the presence of the fungus either within or behind the cicatrice.

The cicatrice formed as a result of wounding (Fig. 4) is very similar in its general characters to that just described. The cells of the phelloderm

as shown in the illustration are very large but there is such a variation in this respect in the different sections that one cannot consider this a constant character of wounded leaves.

Discussion

The evidence presented in the foregoing pages shows that the species of *Prunus, Pyrus,* and *Beta* studied may be expected to react, when attacked by necrogenic pathogenes, by the formation of a cicatrice which isolates the infected portion from the healthy tissue. The character of this cicatrice and the extent of the healing tissue differs with the relation of the infected portion to veins and islet borders. Undoubtedly there is also variation with the age of the leaf at the time at which infection occurs. It is also possible, in fact quite probable, that environmental factors play an important part not only in the character of the cicatrice formed but in the initiation of the process as well.

The character of the cicatrice, with certain minor differences, is essentially the same in all of the species studied. The most striking features of this structure are the renewal of the meristematic condition in the mature mesophyll cells which results in a multiplication of cells in this region, the occlusion of intercellular spaces, and the formation of a definite wound periderm with its phellogen layer which is active in the production of cork cells. These cork cells are frequently greatly enlarged. This enlargement of a layer of cells about the "shot-hole" in leaves of *Prunus sp.* is pointed out by Higgins (7), although he makes no reference to their origin from a cork cambium. That such a cambium may be formed in diseased leaves of *Prunus amygdalus* has been clearly shown by Samuel (17), and a similar condition is reported by McWhorter (12) on diseased leaves of *Carica papaya.*

The experiments with wounded leaves indicate that in the species studied the natural reaction of the suscept to injury is the formation of a cicatrice. Such a cicatrice is similar in its general characters to that formed at the edge of necrotic lesions caused by pathogenic organisms. That the formation of a cicatrice of some nature, as a result of mechanical injury, is of common occurrence in the leaves of many higher plants is evident from the work of Blackman and Matthaei (1), Wyneken (24), Wylie (23), Woit (20), and others.

In the present work no attempt was made to study the development of the healing tissue nor the factors influencing its formation. The work of Samuel (17) is interesting in this connection and deals in considerable detail with the development of this healing tissue in *Prunus amygdalus,* while the work of Blackman and Matthaei (1) is of equal interest in connection

with the formation of healing tissues in leaves subjected to traumatic stimulation.

The fact that in no case where a typical wound periderm is formed about the edge of the lesion was it possible to find any evidence of the hyphae beyond this point would indicate that this periderm was an effectual barrier against the advance of the fungus. This is not conclusive proof, however, as certain physiological factors may be involved and, furthermore, McWhorter (12) has shown that this layer may be penetrated to a slight extent by *Cladosporium papayae*.

Diseases in which no Definite Cicatrice is Formed about the Edge of the Lesion

By far the larger number of the diseases studied were found to fall in this group. The species of pathogenes involved belong to several genera and occur upon a wide variety of suscepts among the flowering plants.

As there seems to be no correlation either between the species of suscept or the species of pathogene and the reaction of the suscept to the advance of the organism, the observations have been grouped according to the genus to which the pathogene belongs.

Lesions produced by Septoria petroselini Desm. on Apium graveolens L. Both the upper and the lower epidermis of the leaf of *Apium graveolens* are of medium thickness and are made up of rather large cells. The palisade parenchyma consists of a single row of rather short broad cells somewhat openly arranged. The spongy parenchyma is made up of cells which vary greatly in both shape and size, some being comparatively small and more or less globose in outline, while others are large and elongated.

The lesions caused by the fungus are small but often coalesce to form large, irregular areas. The main body of the lesion is holonecrotic in character and brownish-gray in color. This holonecrotic area is surrounded by a yellowish-green plesionecrotic zone which is variable in width.

A study of a cross-section through one of the older lesions (Fig. 5) reveals the fact that the cell contents have entirely disappeared and the walls have collapsed. The cell walls are brown, and there is an abundance of intercellular mycelium. The fungous hyphae are plainly visible in the plesionecrotic area and extend to the margin of normal leaf tissue. Those cells in the region of the tips of the advancing hyphae are apparently unchanged but in the intervening cells the contents are broken down and this condition is more complete nearer the necrotic area. In no case is there any evidence of cicatrice formation.

Lesions produced by Septoria podophyllina Pk. on Podophyllum peltatum L. In the healthy leaf of *Podophyllum peltatum* both the upper and

lower epidermis are composed of rather large, irregular, thin-walled cells. There is no well-defined palisade parenchyma but there is a layer of irregularly shaped, somewhat loosely arranged cells, lying just under the upper epidermis. The spongy parenchyma is open, particularly in the lower portion where the globose to elongate cells are arranged in chains, or groups, to enclose large air spaces.

On the material collected the lesions vary greatly in size and are more or less irregular to angular in outline. In many cases this irregularity in outline is due to veins at the edge of the lesion. The lesion is composed of a brown holonecrotic area surrounded by a yellowish-green plesionecrotic zone of varying width. This may be sharply delimited from the normal green tissue of the leaf or may blend gradually into it.

In the necrotic area the cell contents have disappeared and the walls have collapsed. The intercellular hyphae are abundant in this region.

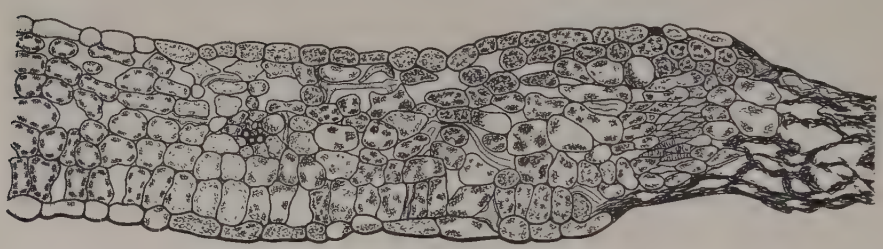

FIG. 5. Cross section through a diseased leaf of *Apium graveolens* showing the histologic changes induced by *Septoria petroselini*. ×171.

The hyphae extend through the plesionecrotic zone and at the outside limit of growth apparently lie between normal cells.

Artificial wounds were made in leaves of *Podophyllum peltatum*. There is no formation of a wound periderm such as is found in *Prunus sp.* but there are certain changes in the character of the contents and in the walls of those cells adjoining the wound. For some distance back from the point of wounding all of the leaf tissues are dead and collapsed, and these collapsed cells contain a brownish substance resembling tannin. Adjoining the inner edge of this collapsed area, and lying between it and normal tissue, there are a few cells which are filled with this brownish substance and whose walls give a positive reaction for suberin. In some cases these cells also may be partly collapsed. There is apparently no increase in either number or size of cells in this region.

Lesions produced by Septoria conspicua Ell. et Mart. on Steironema ciliatum (L.) Raf. The structure of the healthy leaf of *Steironema ciliatum* is rather open. The leaf is very thin but the upper epidermis and also the

lower are quite thick as compared with the total thickness of the leaf. Both epidermal layers are made up of large cells. The palisade parenchyma consists of a single layer of short, broad cells rather loosely arranged. The cells of the spongy parenchyma vary considerably both in size and shape.

On the leaves of this suscept the lesions are circular in outline and rather uniform in size. The central portion of the holonecrotic area is dark brown in color while the margin is a very dark brown. This area is surrounded by a pale yellowish plesionecrotic zone.

The diseased portion is very little thinner than is the normal leaf. In the holonecrotic area the epidermal cells have only partially collapsed. Many of the palisade cells in this region, as well as some of the cells of the spongy parenchyma, are completely filled with a dense granular substance which did not react to any of the stains used. These cells are only slightly shrunken, and in many parts of this area the intercellular spaces are completely filled with the large hyphae. In the plesionecrotic zone the cells of the mesophyll are filled with the same dense granular substance, and occasional intercellular strands of hyphae can be found. Beyond these granulated cells the tissue is normal. There is no other apparent change in the character of the cells in this region.

Lesions produced by Septoria cirsii Niessl. on Cirsium arvense (L.) Scop. The leaf of *Cirsium arvense* is open in structure. The epidermal cells are large and comparatively thick-walled. There is no well-defined palisade parenchyma but there is a layer of loosely arranged, irregular shaped cells lying just beneath the upper epidermis. The remainder of the mesophyll tissue is quite open, the cells varying greatly in both size and shape and arranged more or less in rows, or groups, enclosing large air spaces.

The lesions on the leaves are somewhat irregular in size and shape. The main portion of the lesion is holonecrotic in character, brownish in color, with a well-defined margin. This is surrounded by a narrow, greenish-yellow, plesionecrotic zone. These lesions frequently coalesce to form large irregular patches.

In the holonecrotic area the cell contents have completely disappeared, the cells have collapsed, and the leaf is much thinner at this point. Threads of hyphae are visible between these cells and also in the intercellular spaces of the plesionecrotic zone. The cells comprising this zone have their contents largely disorganized and the walls are partially collapsed. In no case was there any evidence of cicatrice formation.

Lesions produced by Septoria verbasicola B. et C. on Verbascum blattaria L. The healthy leaf of *Verbascum blattaria* is rather thick and in cross-section the cells of the upper epidermis are seen to be much larger

than those of the lower. In both cases the inner and outer walls are thickened. The palisade parenchyma is made up of two or three layers of rather short, broad cells. Occasionally several of the cells of the upper layer have broken down to form a large cavity. The cells of the spongy parenchyma are somewhat irregular in shape and rather compactly arranged.

The lesions in this suscept are variable in size, more or less oval in outline, and frequently coalesce. The holonecrotic center is grayish-white in color while the plesionecrotic area surrounding it is purplish-red and tends to blend into the normal green of the leaf.

In the holonecrotic area the cell contents have entirely disappeared and the walls are completely collapsed. The hyphae are abundant between these collapsed cells, can be seen in the intercellular spaces in the plesionecrotic area, and extend to the edge of normal tissue. The nuclei and chloroplasts have disappeared from most of the cells in this area, some being entirely empty although they still retain their shape. There is no multiplication of cells nor change in the character of the cell walls about the margin of the lesion.

Lesions produced by Septoria acerina Pk. on Acer pennsylvanicum L. The healthy leaf of *Acer pennsylvanicum* is comparatively thin and many of the cells of both the upper and lower epidermis are filled with a dense tannin-like substance. The palisade parenchyma is made up of a single layer of much elongated cells which are closely packed together. The spongy parenchyma is rather open in structure and is composed of cells which vary greatly in size and shape.

The lesions caused by this fungus are small, irregular in outline, and frequently coalesce to form larger areas. The holonecrotic center is dark brown in color and is surrounded by a broad, light-colored, clearly defined plesionecrotic zone.

With an occasional exception, the contents have entirely disappeared from the cells in the necrotic area. The epidermal cells and the cells of the spongy parenchyma have collapsed. Many of the palisade cells have also collapsed and those that have not are very much shortened. The intercellular hyphae are abundant throughout the whole of this area and extend through the plesionecrotic zone to the edge of normal tissue. The contents of the cells in this region are somewhat disorganized but apparently no shrinkage has taken place. Occasional palisade cells, or even groups of two or three of these cells, have densely granular contents. In no case was there any indication of a change in the character of the cell walls except that common to the necrotic area.

Lesions produced by Septoria osmorhizae Pk. on Osmorhiza longistylis (Torr.) D. C. The leaf of *Osmorhiza longistylis* is very thin. The upper

epidermal cells are large and oval to elongate in shape, while those of the lower epidermis are uniformly narrow and much elongated. There is no well-defined palisade parenchyma. The cells of the spongy parenchyma vary from oval to elongate in shape and are arranged parallel to the epidermis.

The lesions on the leaf are small and ovate to angular in outline. The central holonecrotic portion is grayish-white in color while the plesionecrotic zone surrounding it is somewhat darker.

The intercellular hyphae are abundant in the holonecrotic area. The cell contents in this portion of the leaf have entirely disappeared and the cell walls have collapsed, resulting in a tangled mass of cell walls and hyphae. Where there are no large veins the hyphae can be traced for some distance into the plesionecrotic zone and the tips of the advancing hyphae are not more than two or three cells removed from normal tissue. The cells of this region are in various stages of disorganization, depending on the distance they are removed from the holonecrotic area. In no case was there any evidence of cicatrice formation.

Lesions produced by Cercospora caulophylli Pk. on Caulophyllum thalictroides (L.) Michx. The leaf of this suscept is very thin. The upper epidermal cells vary greatly in size and shape, some being very long with the outer and inner walls almost parallel, while others are oval to almost globose. The outer walls are slightly thickened. Except that the cells are smaller and somewhat thinner-walled, the lower epidermis is similar in its structure. The palisade parenchyma consists of a single layer of short, rather broad cells, more or less rectangular in outline. The entire contents of these cells stain deeply with haemotoxylin. The spongy parenchyma cells are globose to elongate in shape and are loosely arranged in such a manner as to enclose large air spaces.

The lesions in these leaves vary in size and are somewhat angular and irregular in outline. The holonecrotic area is dark brown in color, although in many of the larger lesions this may be a lighter brown in the center. The margin is clear-cut and is surrounded by a yellowish-green plesionecrotic zone of varying width. In some cases the lesions may coalesce to form large, irregular-shaped areas.

A study of a cross-section through one of these lesions shows that in the holonecrotic area the epidermal cells and the cells of the spongy parenchyma have completely collapsed while in the cells of the palisade parenchyma this collapsed condition is only partial. In the plesionecrotic zone the cell contents are more or less disorganized but the walls have not collapsed. In this region the intercellular hyphae can be traced to within two or three cells of normal tissue. Apparently there is no histologic reaction which would in any way hinder the advance of the pathogene.

Lesions produced by Cercospora symplocarpi Pk. on Symlocarpus foetidus (L.) Nutt. The upper epidermal cells of *Symplocarpus foetidus* are large and for the most part much longer than broad, with the outer walls fairly straight and the inner ones curving strongly downwards. Both the outer and radial walls are slightly thickened. The lower epidermis is similar to the upper except that the walls are scarcely thickened. The palisade layer is open in structure and consists of a single layer of very short, broad, rectangular cells. Just beneath the palisade layer and making up a part of the spongy parenchyma are two or three rows of cells varying in size and shape but placed for the most part with their long axes parallel to the epidermis. The remainder of the spongy parenchyma is made up of chains of thin-walled cells, irregular in shape and size, and arranged in such a manner as to enclose large air spaces. These cells contain relatively few chloroplasts.

The lesions on the leaf are large, oval in shape, and irregular in outline save where they are bounded by veins. The holonecrotic center is brown in color, shading into the dark brown or black plesionecrotic border. This border is often somewhat raised and is sharply outlined.

A study of a cross-section through one of these lesions (Fig. 6) shows a general collapse of the cells in the holonecrotic area. The plesionecrotic zone is very narrow and consists at most of three or four cells. The nuclei and chloroplasts have largely disappeared from these cells but apparently there has been no shrinking of the walls. In some cases the upper epidermal cells for some distance beyond the necrotic area have collapsed but the cells beneath are apparently normal. No trace of the hyphae was found beyond the holonecrotic region.

In the case of artificial wounding, the cells a short distance back from the wound have entirely collapsed. At the inner edge of this area there is a slight enlargement of the cells of the palisade parenchyma and of those cells lying just beneath it. The walls of these enlarged cells have become suberized, as have also the walls of the epidermal cells bordering upon this area.

Lesions produced by Cercospora menispermi Ell. et Hollw. on Menispermum canadense L. The leaf of *Menispermum canadense* has a heavy upper epidermis composed of large cells with much thickened outer walls. The lower epidermis is also made up of large cells but the outer walls are relatively thin. The palisade parenchyma is made up of a single layer of closely packed, elongated cells and a second layer which is more loosely arranged. The spongy parenchyma is very open with large irregular air spaces.

The brownish lesions on the leaf are somewhat irregular in shape and

size and are largely holonecrotic in character. The narrow plesionecrotic margin is purplish-black in color and well-defined.

In the holonecrotic area the leaf is somewhat thinner than normal. This is due to the collapse of the epidermal cells and those of the spongy parenchyma. The cells of the palisade parenchyma are much reduced in length and are either empty or partially filled with a brownish substance. The intercellular hyhpae are abundant in this region but apparently do not extend into the plesionecrotic zone. There is no change in the character of the cells at the edge of the lesion indicating the formation of a cicatrice.

Lesions produced by Cercospora circumcissa Sacc. on Prunus serotina Ehrh. The upper epidermis of *Prunus serotina* is somewhat thicker than is the lower but otherwise they are very similar. Both the outer and inner walls of these cells are considerably thickened. The palisade parenchyma

FIG. 6. Cross section through a diseased leaf of *Symplocarpus foetidus* showing the histologic changes induced by *Cercospora symplocarpi*. ×197.

consists of a single layer of cells which are somewhat elongated and taper slightly toward the lower end. These cells are rather compactly arranged, although there are evident intercellular spaces between many of them. The cells of the spongy parenchyma are irregular in size and shape and are so arranged that they enclose large air spaces.

Lesions produced on this suscept are angular in outline and quite variable in size. The holonecrotic portion varies in color from a light brown to a gray and is bordered by a reddish-brown plesionecrotic zone.

In the holonecrotic area the leaf is somewhat thinner than normal. The contents of the cells of the spongy parenchyma have disappeared and the walls have collapsed. This is also the case in many of the palisade cells, but some are partially filled with a dense granular substance. The hyphae are not at all plentiful in any part of the lesion, but occasional threads are

visible between the cells in both the holonecrotic and the plesionecrotic areas. The contents of the cells of the latter region are more or less disorganized. There was no evidence of cicatrice formation at the edge of the lesion.

Lesions produced by Ramularia primulae Thüm. on Primula polyantha Mill. The upper epidermis of *Primula polyantha* is much thicker than is the lower. It is made up of much larger cells, the outer walls of which are markedly thickened. Occasional cells in both of these layers are filled with a dense, tannin-like substance. The palisade parenchyma is made up of a single layer of short thick cells, loosely arranged. The spongy parenchyma is composed of cells of various sizes and shapes and is moderately open in arrangement.

The lesions on the leaf of this suscept are variable in size and somewhat irregular in outline. The holonecrotic center is grayish in color with a sharply defined somewhat darker margin. Surrounding this area there is a rather broad yellowish-green plesionecrotic zone. The lesions are numerous upon the leaf and often unite to form large necrotic patches.

In the holonecrotic area the cell contents have broken down and disappeared. The cell walls have collapsed to a large extent, leaving the leaf somewhat thinner than normal at this point. In the outer edge of this region the epidermal cells are collapsed (Fig. 7) and the contents of the mesophyll cells are in various stages of disorganization. The intercellular hyphae, although not abundant in the holonecrotic area, are plainly visible and extend through the plesionecrotic zone to a point within a few cells of normal tissue. In the plesionecrotic zone none of the cells are collapsed. In the mesophyll cells of this region the nuclei and chloroplasts have disappeared and the cytoplasm is partially broken down. There is no evidence of any healing tissue at or near the edge of the lesion which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia ranunculi Pk. on Ranunculus recurvatus Poir. The leaf of *Ranunculus recurvatus* is extremely open in structure. The upper and lower epidermis are very similar in structure and are composed of rather irregularly shaped cells whose outer walls are slightly thickened. There is no well-defined palisade parenchyma, but the irregularly shaped cells which compose the mesophyll are more closely arranged in that region.

The lesions on the leaves of this suscept are large, circular to oval in outline except where they are bounded by large veins. They are entirely holonecrotic in character and brownish in color with somewhat darker centers.

In the center of the diseased area the contents of all of the cells have disappeared and the walls have collapsed, thus causing the leaf to be much

thinner than normal at this point. Farther out toward the edge of the lesion the epidermal cells may or may not be collapsed and the leaf gradually increases to normal thickness although the contents of the mesophyll cells may be entirely disorganized and the walls partially shrunken. The threads of hyphae can be seen in the intercellular spaces of the mesophyll and extend to the very edge of normal tissue. There is no evidence of any suscept reaction of any kind which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia decipiens Ell. et Ev. on Rumex crispus L. The leaf of *Rumex crispus* is very open in structure. The cells of the upper epidermis contain a dense granular substance in the protoplasmic layer, and in some cases the cells are completely filled with this material. The palisade parenchyma is composed of a single layer of rather short, broad

FIG. 7. Cross section through a diseased leaf of *Primula polyantha* showing the histologic changes induced by *Ramularia primulae*. ×187.

cells, loosely arranged. The cells of the spongy parenchyma vary widely in size and shape.

The lesions vary greatly in size and are more or less irregular in shape. The central part of the holonecrotic area is light brown in color with a wide, somewhat darker-colored margin. This is usually surrounded by a purplish plesionecrotic zone.

The holonecrotic area is very little thinner than normal. The cell contents in this part of the leaf have entirely disappeared. The epidermal cells, and to a certain extent the cells of the spongy parenchyma, have collapsed. The hyphae are intercellular and can be traced throughout the plesionecrotic zone to the very edge of normal tissue. In this zone the cells of the epidermis and the mesophyll are in various stages of disorganization and collapse. There is no evidence of histologic changes which would tend to prevent the advance of the pathogene.

Lesions produced by Alternaria solani (E. et M.) Jones et Grout on Solanum tuberosum L. The structure of the healthy leaf of *Solanum tuberosum* is rather open. The upper epidermis is much thicker than the

lower and the cells are more or less filled with some granular substance. The outer walls of the cells of both the upper and lower epidermis are slightly thickened. The cells of the palisade parenchyma are narrow, much elongated, and irregular in outline with large air spaces between the individual cells. The cells of the spongy parenchyma are irregular in shape, and the air spaces in this tissue are also large.

The lesions on this suscept are entirely holonecrotic in character, irregular in shape and size, somewhat angular in outline and marked with concentric rings. The color is dark brown and the margin is well defined.

In the diseased area the upper epidermal and palisade cells are filled with a dense granular substance (Fig. 8). The cells of the spongy paren-

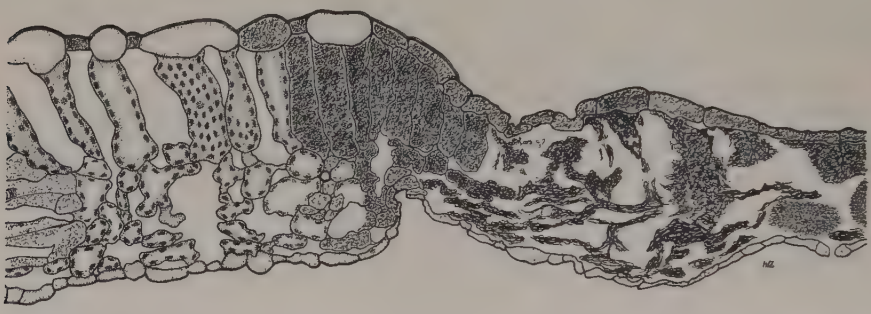

FIG. 8. Cross section through a diseased leaf of *Solanum tuberosum* showing the histologic changes induced by *Alternaria solani*. ×145.

chyma are collapsed, as are also the cells of the lower epidermis. The palisade cells are much reduced in length and partially collapsed except in the region of the concentric rings where they stand out more prominently. The necrotic area ends almost abruptly in normal tissue, although the cells on the outer edge have not yet collapsed. The hyphae are abundant between the shrunken mesophyll cells, particularly in the spongy parenchyma, and can be traced almost to the edge of the normal tissue. There is no evidence of cicatrice formation.

Lesions produced by Alternaria solani (E. et M.) Jones et Grout on Lycopersicum esculentum Mill. The general structure of the leaf of *Lycopersicum esculentum,* the appearance of the lesions, and the reaction of the suscept to the invasion of the pathogene are almost identical with those just described for *Solanum tuberosum.*

Lesions produced by Macrosporium cucumerinum Ell. et Ev. on Cucumis melo L. The leaf of this suscept is quite thick. Both the lower and upper epidermal cells are large and their outer walls are slightly thickened. The palisade parenchyma is made up of a single layer of narrow, much

elongated cells, rather closely arranged. The cells of the spongy parenchyma are variable in shape and size and are arranged somewhat loosely.

The lesions caused by this pathogene are variable in size, regular in outline except where they are bounded by veins, and frequently coalesce to form large necrotic areas. The holonecrotic center is light brown in color. A narrow plesionecrotic zone surrounds this area but is more marked on the under surface of the leaf than on the upper.

In the holonecrotic area the leaf is much thinner than normal, the cell contents have disappeared, and the walls have collapsed. This collapsing of the walls is more complete in the cells of the spongy parenchyma. These shrunken cells are interwoven with the intercellular hyphae and they are present in the plesionecrotic zone, extending to a distance of one or two cells from normal tissue. The contents of the cells in this zone are partially broken down but the walls have not collapsed.

Lesions produced by Phyllosticta fraxinii Ell. et Mart. on Fraxinus americana L. In the leaves of *Fraxinus americana* the upper and lower epidermis are quite similar in structure except that in the former the cells are filled with some dense granular substance. The palisade parenchyma is made up of a double layer of narrow, elongated cells which are arranged to form a compact wall. The cells of the spongy parenchyma vary in size and shape and are rather closely arranged, the intercellular spaces being comparatively small.

The lesions on this leaf are large and more or less irregular in outline. The main body of the lesion is holonecrotic in character and light brown in color. It is surrounded by a purplish-black plesionecrotic zone of varying width.

In the holonecrotic area the epidermal cells and the cells of the spongy parenchyma are empty and collapsed. The palisade cells are slightly reduced in length, somewhat shrunken, and partially filled with some dense granular material. The leaf at this point, however, is not much thinner than normal. The contents of the cells in the plesionecrotic zone are somewhat disorganized. The hyphae are visible between the cells in this region and apparently advance to within two or three cells of healthy tissue.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago sativa L. Both the upper and lower epidermis of *Medicago sativa* are similar in structure, the cells varying considerably in size and shape, with the outer walls markedly thickened. The palisade cells are arranged in one or two layers, some being rather short and broad while others are much elongated. They are somewhat open in arrangement. The cells of the spongy parenchyma are also loosely arranged and vary greatly both in size and shape.

The lesions are numerous and scattered on the leaves. They appear as small holonecrotic areas which are dark purple in color and surrounded by a yellowish plesionecrotic border which blends into the normal green of the leaf.

In the holonecrotic area the palisade cells are almost entirely replaced by the stroma of the fruiting body while the cells of the spongy parenchyma are either collapsed or filled with the hyphae. In the plesionecrotic zone the epidermal cells may be collapsed. The hyphae can frequently be seen in one or more cells beyond this point and occasional threads of hyphae can be seen in the mesophyll cells of this region and may extend to within two or three cells of normal tissue. The cells in this zone have their contents more or less disorganized and an occasional palisade cell is filled with some dense brownish substance, but there is little if any collapsing of the cell walls. There is no evidence of cicatrice formation.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago lupulina L. The structure of the leaf of *Medicago lupulina* differs from that of *Medicago sativa* in being very much thinner. This difference in thickness is mainly due to the shorter palisade cells but is also due to some extent to a thinner layer of spongy parenchyma.

The lesions on this suscept are very similar in their general characters to those described on *Medicago sativa.* The suscept reactions and the extent of the mycelial invasion are in all respects similar to those just described for *Medicago sativa.*

Lesions produced by Pseudopeziza trifolii (Bernh.) Fckl. on Trifolium pratense L. The leaf of *Trifolium pratense* resembles that of *Medicago sativa* in its general structure. The palisade cells are not so long, however, and although the leaf blade is approximately the same thickness in the two species, the lack in length of palisade cells is made up by a corresponding increase in thickness of spongy parenchyma in *Trifolium pratense.*

In general the character of the lesions on the leaves is similar to that given for the lesions on leaves of *Medicago sativa.* The suscept reactions and the extent to which the hyphae have penetrated the leaf tissues are the same as have been described in connection with *Medicago sativa.*

Lesions produced by Pseudopeziza ribis Kleb. on Ribes grossularia L. The leaf of *Ribes grossularia* is rather thick. The upper epidermis differs from the lower in having larger cells whose walls are somewhat thicker. The palisade parenchyma consists of two or even three layers of cells. These cells are quite broad and those of the upper layer are much longer than those of the lower. They are closely packed together. The cells of the spongy parenchyma are variable in size and shape. They are arranged in chains or groups and, while only moderately open in arrangement, the intercellular spaces are at times quite large.

The lesions on this suscept are small, more or less round in outline with an indefinite margin. The color is a dark purple with a grayish center in the older lesions.

The condition existing here as a result of the invasion of the pathogene is so similar to that already described for *Pseudopeziza medicaginis* that no further description is necessary.

Lesions produced by Rhytisma ilices-canadensis Schw. on Ilex verticillata (L.) Gray. A study of the leaf of *Ilex verticillata* shows the upper epidermal cells to be very regular in shape and size, almost rectangular in outline, and with a heavy outer wall. The lower epidermal cells are not so regular either in shape or size as those of the upper epidermis, nor are the outer walls quite so thick. The palisade parenchyma consists of a single layer of cells. These are narrow, much elongated, very regular in shape, and closely arranged. The cells of the spongy parenchyma are irregular in size and shape and so arranged as to leave rather large air spaces between the cells or groups of cells.

The lesions on the leaves are irregular in size and shape. The holonecrotic portion is black in color and is surrounded by a narrow, light green plesionecrotic border which blends into the normal green of the leaf.

When viewed in cross-section the black portion of the lesion is seen to consist of a stroma with a very dense black outer covering. On the upper surface of the leaf this stroma is only slightly elevated and beneath it the palisade cells are still in evidence, although they have shrunken and the contents have become disorganized. The hyphae are apparently intercellular. On the lower surface of the leaf the stroma is very much thicker and in some cases may cover a larger area than does the upper stroma. The contents of the spongy parenchyma cells which lie between the two stromata are usually wholly disorganized. Where the lower stroma covers a larger area than the upper, those parenchyma cells lying next to the stroma are disorganized while the remaining mesophyll cells may be perfectly normal. The hyphae do not appear to extend into the plesionecrotic zone and the contents of the cells in this region are only partially disorganized. There are apparently no histologic changes in the suscept which would prevent the further advance of the pathogene.

Lesions produced by Rhytisma ilices-canadensis Schw. on Nemopanthus mucronata (L.) Trel. Although similar in its general structure, the leaf of *Nemopanthus mucronata* is much thinner than that of *Ilex verticillata* and the upper epidermal cells are partially filled with a dense material resembling tannin.

The lesions are very similar in appearance to those just described on *Ilex verticillata* and in general the suscept reaction is the same. In the

sections examined, however, the hyphae extend a short distance into the plesionecrotic zone.

Lesions produced by Rhytisma acerinum (Pers.) Fr. on Acer rubrum L. The general structure of the leaf of *Acer rubrum* is the same as that of *Nemopanthus mucronata.* The appearance of the lesions and the type of suscept reaction are very similar to those described for *Ilex verticillata.*

Lesions produced by Mycosphaerella fragariae (Tul.) Lind. on Fragaria virginiana Duch. In the healthy leaf of this suscept the cells of both epidermal layers are large and rather broad. Many of the cells of the upper epidermis are partially filled with a material resembling tannin. The palisade parenchyma is composed of a single layer of compactly arranged cells which are rather short and taper slightly towards the lower end. The cells of the spongy parenchyma are irregular in size and shape and are very loosely arranged.

The lesions on the leaf are somewhat irregular in size and shape. In the older lesions the center of the holonecrotic area is grayish in color with a reddish-purple margin. This is surrounded by a wide plesionecrotic zone which is also reddish-purple in color, blending into the normal green of the leaf.

In the center of the holonecrotic area the cells have collapsed and the leaf is somewhat thinner than normal at this point. In the outer edge of this area the cells are filled with a brownish substance resembling tannin which failed to react to any of the stains used. These cells are not collapsed. In the plesionecrotic zone all of the epidermal cells are filled, or at least partly filled, with some material which stains deeply with haemotoxylin. The contents of the mesophyll cells of this region stain more intensively than do the normal cells. The chloroplasts, although present, appear to be degenerating. The hyphae are present between the cells in the holonecrotic area but no trace of them can be found beyond the zone of mesophyll cells which are filled with the tannin-like substance. In no case is there any evidence of cicatrice formation although occasional cells at the outer edge of the holonecrotic area may be slightly enlarged.

When leaves of *Fragaria virginiana* are wounded a cicatrice is formed about the edge of the wounded portion. Multiplication and enlargement of the mesophyll cells occurs. All of these cells are thick-walled and the walls of the outer row are suberized. There is no evidence of a wound periderm such as occurs in *Prunus sp.*, but all of the cells are filled with the same granular substance found at the edge of the lesions produced by *Mycosphaerella fragariae.*

Lesions produced by Mycosphaerella grossulariae (Fr.) Lind. on Ribes prostratum L'Her. In a healthy leaf of *Ribes prostratum* the upper epi-

dermis is somewhat thicker than the lower. The walls of the upper epidermal cells are considerably thickened, and this thickening is present to a lesser extent in the walls of the lower epidermal cells. The palisade parenchyma is well-defined and is made up of a single layer of elongated cells closely packed together. The spongy parenchyma is composed of cells of various shapes and sizes which are loosely arranged.

The lesions on this suscept are small and rather regular in outline. The holonecrotic center is grayish-white in color and is bordered by a narrow purplish-black plesionecrotic zone.

The holonecrotic portion is very little thinner than is the normal portion of the leaf. In this area many of the cells, both in the palisade and in the spongy parenchyma, have become filled with a dense granular substance resembling tannin. Such cells have only partially collapsed. All other cells in this area are apparently empty. The epidermal cells are collapsed and this condition is quite common even in the plesionecrotic zone. The contents of the mesophyll cells in this zone are becoming disorganized and the cells are filling up with the dense material already referred to. The intercellular hyphae extend for a short distance into the plesionecrotic zone. There is no evidence of the formation of a healing tissue about the edge of the lesion.

Lesions produced by Sphaerella tussilaginis Rehm. on Tussilago farfara L. In the healthy leaf of *Tussilago farfara* the upper epidermis is much thicker than the lower, the cells are larger, and the outer walls are much thicker. While the upper epidermal cells are arranged above the palisade cells in the ordinary way, the lower epidermis is decidedly wavy in its appearance. It appears as a chain of cells attached to the spongy parenchyma at regular intervals, each loop of the chain forming the outer boundary of a large air space. The palisade parenchyma is composed of about four layers of cells, the cells of the upper layer being much shorter and more regular in shape than those of the lower layers. These cells are closely packed, no intercellular spaces being visible in cross section of the leaf. The cells of the spongy parenchyma vary from almost square to very narrow and much elongated and are arranged in chains or groups. To the lower part of these chains, or groups of cells, the lower epidermis is attached.

The lesions on the leaf are circular in outline except when they are bounded by large veins. In the older lesions the holonecrotic center is grayish in color, and this is surrounded by a dark purple plesionecrotic zone of varying width. The edge of the lesion is not well-defined.

In the holonecrotic area the cells are entirely empty and the cell walls have collapsed. The leaf is about one half its normal thickness at this point.

In the plesionecrotic zone the chloroplasts have broken down and disappeared but otherwise there seems to be little disorganization of the cell content. The upper layer of palisade cells and the cells of the upper epidermis contain some substance which stains deeply with haemotoxylin. No trace of the fungus was found outside of the holonecrotic area nor was there any evidence of cicatrice formation.

Lesions produced by Gnomonia leptostyla Fr. on Juglans cinerea L. The leaf of *Juglans cinerea* is very thin. The majority of the cells of the upper and all of those of the lower epidermis are narrow, long, and irregular in outline. In the upper epidermis, however, are found either single cells or groups of cells which are much larger than the others and either globose or oval in shape. The palisade parenchyma consists of a single layer of cells which are rather short and slightly narrowed at the lower end. The spongy parenchyma is made up of two or three layers of loosely arranged cells. The cells of the upper layer are large and resemble those of the palisade layer while the cells of the lower layers are very narrow and elongated.

The lesions on the leaf are somewhat variable in size and frequently unite to form large necrotic areas. The holonecrotic area is brownish-gray in color. The extreme margin of this area is really plesionecrotic in character.

In the holonecrotic area the leaf is very little thinner than normal. The epidermal cells and those of the spongy parenchyma are empty and the walls have collapsed. The palisade cells are partially filled with some dense granular material. These cells have apparently been reduced in length but the walls have not collapsed to the same extent as have the walls of the other mesophyll cells. In the plesionecrotic zone the contents of the cells are only partially disorganized. The hyphae are not abundant in the diseased region, but occasional threads are visible between the cells in the holonecrotic area and these extend to some extent into the plesionecrotic zone. This zone blends gradually into normal tissue.

Lesions produced by Guignardia bidwellii (Ell.) V. et R. on Ampelopsis tricuspidata Planch. In the healthy leaf of *Ampelopsis tricuspidata* the upper epidermis is composed of large cells which are, for the most part, somewhat oval in outline. The outer walls are very thick and the radial and inner walls, while somewhat thinner, are relatively thick as compared with the walls of the mesophyll cells. The cells of the lower epidermis are more irregular in size than those of the upper and the walls are markedly thickened. The palisade parenchyma is well-defined and is made up of two layers of cells. Those of the upper layer are long, narrow, and closely packed, while those of the lower layer are considerably shorter, somewhat

broader and more loosely arranged. The cells of the spongy parenchyma are variable both in size and shape. Some are almost rectangular in outline, others are almost globose, while still others are narrow and much elongated. These cells are rather loosely arranged and the intercellular spaces are fairly large.

The lesions on the leaves are variable in size and are usually irregular in shape. They frequently coalesce to form large necrotic areas and in extreme cases the greater part of the leaf may be affected. The holonecrotic center is brown in color with a sharply defined, lightly raised, purple-black margin which is plesionecrotic in character.

In the holonecrotic area the palisade cells have not collapsed completely but are much reduced in length. Many of these cells are filled with a dense granular material resembling tannin. The cells of the spongy parenchyma are for the most part empty and shrunken. In the plesionecrotic zone the cells have lost their chloroplasts although the cytoplasm is still in evidence (Fig. 9). In this region the cells of the upper layer of palisade parenchyma are slightly longer than normal and the upper epidermal cells are greatly enlarged. In the latter cells the contents have either disappeared or they are densely granular. The remainder of the mesophyll cells and those of the lower epidermis are apparently normal in size although their contents are somewhat broken down. As considerable difficulty was experienced in staining the mycelium, no satisfactory evidence was secured relative to the abundance of the hyphae in the holonecrotic area nor the distance to which it had penetrated the plesionecrotic zone.

Artificial wounding of the leaves of this suscept resulted in the formation of a definite cicatrice (Fig. 10). This cicatrice is of the same general type as that produced on the leaves of *Prunus domestica* when similarly treated.

Discussion

While the leaf tissues of the suscepts included in this group do not respond to the invasion of the pathogene by the formation of a definite cicatrice, nevertheless the cells about the holonecrotic area are far from being normal in character. The changes occurring in these cells are mainly disorganization of the protoplasm and the collapse of the cells. The extent or degree of such changes varies in different plants and seems to be associated with the character of the suscept rather than the pathogene invading the tissues.

In some leaves the cells in the holonecrotic area may be entirely empty and collapsed, a condition found in *Apium graveolens* when attacked by *Septoria petroselini,* and as a result this portion of the leaf may be much

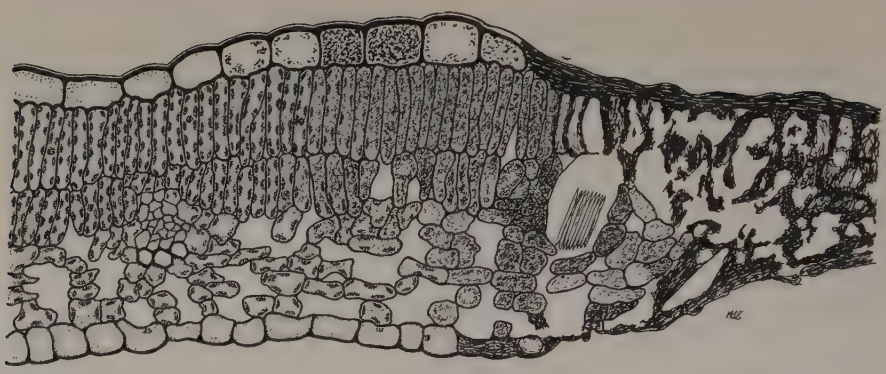

FIG. 9. Cross section through a diseased leaf of *Ampelopsis tricuspidata* showing the histologic changes induced by *Guignardia bidwellii*. ×230.

thinner than normal. On the other hand many of the cells in this area may be partially or wholly filled with a dense granular material as is the case with leaves of *Steironema ciliatum* attacked by *Septoria conspicua*. In such leaves many of the palisade cells and some of the cells of the spongy

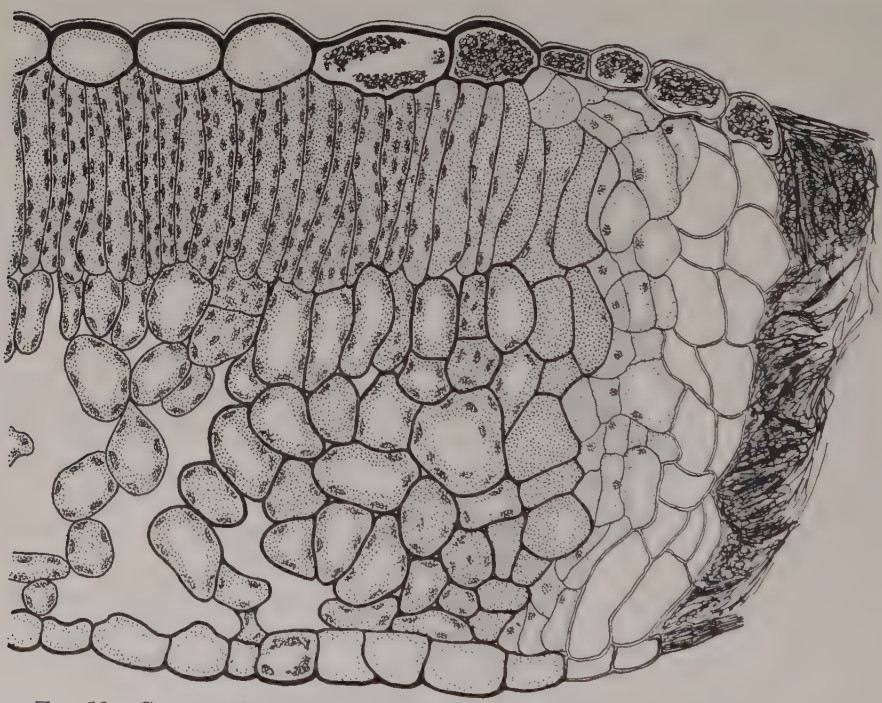

FIG. 10. Cross section through the edge of a wound on the leaf of *Ampelopsis tricuspidata* showing the cicatrice formed under these conditions. ×321.

parenchyma contain this substance and as a result little or no shrinking of the necrotic tissues has occurred.

The extent to which the hyphae of the pathogene penetrate beyond the holonecrotic area differs greatly in the leaves of the plants studied. Not only does this vary with the different plants but it varies in different lesions on the same leaf and is affected in many cases by the presence or absence of veins. The extent of this hyphal invasion is important in its effect upon the cells in the invaded region for such cells invariably show the influence of the pathogene by a disorganization of the protoplasm, followed later by a partial or total collapse of the cells. In leaves of *Apium graveolens* attacked by *Septoria petroselini* the hyphae extend for a long distance beyond the holonecrotic area, as reported by Rogers (16), and the cell contents in this region are more or less disorganized. The same condition is found in leaves of *Primula polyantha* attacked by *Ramularia primulae,* and also in leaves of *Caulophyllum thalictroides* attacked by *Cercospora caulophylli.* On the other hand in leaves of *Symplocarpus foetidus* attacked by *Cercospora symplocarpi* the hyphae extend little if any beyond the holonecrotic area and only a few cells can be classed as plesionecrotic. A similar condition exists in leaves of *Ilex verticillata* attacked by *Rhytisma ilices-canadensis,* in leaves of *Medicago sativa* infected by *Pseudopeziza medicaginis,* and in leaves of *Solanum tuberosum* bearing lesions caused by *Alternaria solani.*

In the case of the last mentioned suscept it was thought that possibly the target-board effect caused by *Alternaria solani* might be due to the formation of a periderm as was shown by Hesler (6) to be the case in *Pyrus malus* attacked by *Sphaeropsis malorum.* Jones (10) and Rands (15) have suggested that this effect is caused by the epidermal cells being thrown into folds due to shrinking caused by the collapse of the mesophyll cells. Whatever may be the effect of this shrinkage of the mesophyll cells the writer's observations show that, for some unknown reason, the palisade cells of the target board lines are only partially collapsed while this collapse is complete in the cells between these lines.

CONCLUSIONS

The results reported in this paper show clearly that whatever may be the factor or factors governing the limitation of the necrosis caused by fungi in the leaf spot diseases of plants, it is by no means due in all cases to the formation of a cicatrice or healing tissue within the suscept, which limits the advance of the pathogene. In some plants the usual reaction of the leaf to pathogene injury appears to be the formation of a cicatrice. In a large majority of cases, however, there is no attempt on the part of the

suscept to lay down such a barrier. Neither does it follow that a plant which forms a cicatrice about mechanical wounds in its leaves will respond in the same way to localized necrosis caused by fungi. On the other hand all of the suscepts studied during these investigations which did produce a cicatrice about a fungous necrosis also formed a cicatrice about mechanical injuries. Whether these plants react in the same way to the attacks of other leaf-spotting organisms remains a problem for future work.

SUMMARY

Leaves of *Prunus domestica, P. avium, P. cerasus,* and *P. virginiana,* when attacked by species of *Coccomyces,* form a definite cicatrice about the edge of the lesion, thus isolating the diseased portion from the healthy. The same reaction occurs when leaves of *Pyrus communis* are attacked by *Mycosphaerella sentina* and when leaves of *Beta vulgaris* are attacked by *Cercospora beticola.* Artificial wounding of leaves of these plants results in the formation of a cicatrice similar to that formed in diseased leaves.

No cicatrice is formed in either diseased or wounded leaves of *Podophyllum peltatum* or *Symplocarpus foetidus.* In wounded leaves of these plants the cells for some distance from the edge of the wound are dead and collapsed while the walls of a few cells adjoining the inner edge of this collapsed area are suberized.

Diseased leaves of *Fragaria virginiana* showed no evidence of cicatrice formation, but wounded leaves of this plant formed a definite cicatrice about the edge of the wound. These cells have thickened walls and are suberized in part while all of the cells are filled with a dense granular material. There is no evidence of a wound periderm such as is formed in wounded leaves of *Prunus domestica.*

Although diseased leaves of *Ampelopsis tricuspidata* do not react by the formation of a cicatrice, yet wounded leaves of the same plant form a well developed wound periderm about the edge of the injury.

Diseased leaves of all other suscepts included in this investigation failed to form a cicatrice separating the lesion from healthy tissues. The type of reaction in these cases consists of a degeneration of the protoplasm, with or without granular deposits, together with more or less collapsing of the cells. No evidence of suberization of the walls of the cells at or near the edge of the lesions was observed. These leaves were not wounded and their reaction to such injuries is unknown.

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